Outdoor and Indoor Cultivation of *Spirulina platensis* in the Extreme South of Brazil

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Water supplemented with 10% or 20% (v/v) of Zarrouk medium was used to cultivate *Spirulina platensis* in closed and open bioreactors under controlled conditions (30 °C, 32.5 μmol m^{-2} s^{-1}, 12 h light/dark photoperiod) and in a greenhouse (9.4 to 46 °C, up to 2800 μmol m^{-2} s^{-1}, variable day length photoperiod) using different initial biomass concentrations (X_0) in the extreme south of Brazil (32.05° S, 52.11° W). Under controlled conditions the maximum specific growth rate (μ_{max}) was 0.102 d^{-1}, the biomass doubling time (t_d) was 6.8 d, the maximum dry biomass concentration (X_{max}) was 1.94 g L^{-1} and the maximum productivity (P_{max}) was 0.059 g L^{-1} d^{-1}, while the corresponding values in the greenhouse experiments were μ_{max} = 0.322 d^{-1}, t_d = 2.2 d, X_{max} = 1.73 g L^{-1} and P_{max} = 0.112 g L^{-1} d^{-1}. Under controlled conditions the highest values for these parameters occurred when X_0 = 0.15 g L^{-1}, while in the greenhouse X_0 = 0.4 g L^{-1} produced the highest values. These results show that the cultivation of *S. platensis* in greenhouses in the extreme south of Brazil is technically viable and that the *S. platensis* inoculum and the concentration of Zarrouk medium can be combined in such a way as to obtain growth and productivity parameters comparable, or superior, to those occurring in bioreactors under controlled conditions of temperature, illuminance and photoperiod.

Key words: Bioreactor, Southern Brazil, *Spirulina platensis*

Introduction

The use of the solar energy to cultivate photosynthetic microorganisms has become of increasing interest to researchers and investors. One of the most studied photosynthetic microorganisms is *Spirulina*, which is produced worldwide due to its nutritional and therapeutic properties and the fact that its dehydrated biomass can be used as a human or animal dietary supplement.

The growth of photosynthetic microorganisms is influenced by factors such as the susceptibility of the culture to evaporation and contamination (Vonshak and Richmond, 1988), type and intensity of agitation (Hosaka *et al*., 1995), temperature, illuminance, duration of the photoperiod, *i. e.* light/dark cycles (Hase *et al*., 2000) and illuminance gradients within bioreactors (Grima *et al*., 1996).

Closed bioreactors facilitate temperature control and reduce, or eliminate, contamination (Travieso *et al*., 2001; Torzillo *et al*., 1986); illuminance can be improved in flat-plate bioreactors (Richmond *et al*., 2003) or when the concentration of cells is low (Chen *et al*., 1997). However, open bioreactors are most often used for the large-scale cultivation of photosynthetic microorganisms (Jiménez *et al*., 2003; Belay, 1997) because such reactors use solar energy for heat and light and are cheap to construct and simple to operate, although the productivity of such systems is generally low.

Open bioreactors exposed to natural environmental conditions are subject to the prevailing climatic conditions that can influence the growth of the target microorganism and can limit cultivation to the hottest months of the year, making it important to evaluate the climatic potential of areas with regard to the production of photosynthetic microorganisms (Jiménez *et al*., 2003; Hase *et al*., 2000). Furthermore, Hase *et al*. (2000) have pointed out that covered bioreactors with a structure like a greenhouse can be a low-cost alternative which can compensate for negative environmental effects such as low temperature.

Nutrient costs are also an important factor in the production of *Spirulina* biomass (Vonshak, 1997), and the reduction of such costs without a decrease in productivity can ensure that culturing

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Spirulina biomass is economically viable. Zarrouk medium (Zarrouk, 1966) was formulated for the cultivation of *Spirulina*; however it has been reported that maximum *Spirulina* biomass productivity occurred in open tank bioreactors using 20% (v/v) Zarrouk medium (Radmann *et al*., 2007).

Several *Spirulina* strains can grow between 12 and 43 °C, and the temperature optimum for growth is between 30 and 38 °C (Belay, 1997; Tomaselli *et al*., 1993). In the extreme south of Brazil, a subtropical area, temperatures within the optimal range for the growth of *Spirulina* usually occur from December to March (summer in the southern hemisphere); in the other months temperatures can be less than 12 °C. The objective of the work described in this paper was to evaluate the cultivation of *Spirulina platensis* in open and closed bioreactors situate in the extreme south of Brazil and to assess the influence of the nutrient concentration and initial *S. platensis* biomass concentration.

Material and Methods

We cultivated *Spirulina platensis* strain LEB-52 (Costa *et al*., 2004) in tap water supplemented with 10% or 20% (v/v) of Zarrouk medium (Zarrouk, 1966). Cultures were accomplished in two sets of bioreactors: in closed 2 L Erlenmeyer flasks with an 1.8 L working volume, agitated by sterile air bubbling; and in 6 L acrylic open raceway bioreactors, agitated by acrylic paddles rotating at 18 revs min^{-1}. For both sets of bioreactors we used two environmental conditions: One set of the bioreactors was placed in a growth chamber at 30 °C, illuminated with 32.5 μmol m^{-2} s^{-1} using 40 W daylight-type fluorescent lamps (Osram, São Paulo, Brazil) under a 12 h light/dark photoperiod (Costa *et al*., 2000); while the other set of bioreactors was placed in an outdoor greenhouse, protected from UV light, and exposed to the natural environmental conditions prevailing in the extreme south of Brazil (32.05° S, 52.11° W) during May and June of 2004. The initial biomass concentration was 0.15 g L^{-1} or 0.40 g L^{-1}, and the cultures were maintained until the death phase or until a maximum of 50 d. The volume of the media in the open bioreactors was maintained constant by the daily replacement of the water lost by evaporation. Each experiment was replicated.

For each bioreactor type the cultures were carried out according to a complete factorial 2^3 design (Box *et al*., 1978) in which the factors were the content of Zarrouk medium (10% and 20% v/v), the initial biomass concentration (X_0 = 0.15 g L^{-1} and 0.4 g L^{-1}) and the environmental conditions (controlled in a growth chamber at 30 °C, 32.5 μmol m^{-2} s^{-1} and 12 h light/dark photoperiod and uncontrolled in a greenhouse under prevailing environmental conditions). The *S. platensis* biomass concentration was determined every 24 h by measuring the optical density of the cultures at 670 nm and comparing the values to previously prepared standard curves of optical density versus biomass (Costa *et al*., 2002). The maximum and minimum temperatures of the air inside the greenhouse were registered daily by a digital thermometer and the illuminance was determined at the surface of the open cultures using a MLM 1332 digital lightmeter (Minipa, Brazil).

At the end of each run the *S. platensis* biomass (X) values were used to calculate the maximum specific growth rate (μ_{max}, d^{-1}) by the exponential regression of the logarithmic growth phase of the curve produced by a plot of biomass versus time. The biomass doubling time (t_d, d) was calculated using natural logarithms (ln) as $t_d = \ln 2/\mu_{max}$ (Bailey and Ollis, 1986). The maximum *S. platensis* biomass concentration (X_{max}, g L^{-1}) was also recorded and the maximum productivity (P_{max}, g L^{-1} d^{-1}) calculated from the equation $P = (X_t - X_0)/(t - t_0)$, where X_t is the biomass concentration (g L^{-1}) at time t (d) and X_0 the initial biomass concentration (g L^{-1}) at t_0 (Schmidell *et al*., 2001). All results were submitted to analysis of variance (ANOVA) at the 90% confidence interval ($p < 0.1$).

Results and Discussion

The highest maximum specific growth rate (μ_{max}) for cultures growing under controlled conditions was 0.102 d^{-1} in open bioreactors containing water supplemented with 10% Zarrouk medium and inoculated with 0.15 g L^{-1} of *S. platensis* biomass, giving a biomass doubling time (t_d) of 6.8 d (Table I). Under controlled conditions and irrespective of the bioreactor type, μ_{max} was always higher for the 10% Zarrouk medium cultures than for the 20% Zarrouk medium cultures (Table I). The initial *S. platensis* biomass concentration of the inoculum (X_0) did not significantly alter the value of μ_{max} in closed bioreactors under controlled conditions, but in open bioreactors μ_{max} de-

Table I. Growth conditions and kinetic and productivity data for *Spirulina platensis* growing in water supplemented with 10% or 20% (v/v) Zarrouk medium in closed bioreactors and raceway bioreactors under controlled conditions (30 °C, 32.5 μmol m^{-2} s^{-1}, 12 h light/dark photoperiod) and in a greenhouse (9.4 to 46 °C, up to 2800 μmol m^{-2} s^{-1}, variable day length photoperiod), initial biomass concentration (X_0) of 0.15 or 0.40 g L^{-1}. The parameters evaluated (mean ± standard deviation) were the maximum specific growth rate (μ_{max}, d^{-1}), biomass doubling time (t_d, d), maximum biomass concentration (X_{max}, g L^{-1}) and maximum productivity (P_{max}, g L^{-1} d^{-1}).

Content (% v/v) of Zarrouk medium (ZM) and inoculum size (X_0 [g L^{-1}])	μ_{max} [d^{-1}]	t_d [d][a]	X_{max} [g L^{-1}]	P_{max} [g L^{-1} d^{-1}]
Controlled conditions, closed bioreactors				
10% ZM, X_0 = 0.15	0.075 ± 0.001	9.2	1.79 ± 0.17	0.056 ± 0.002
20% ZM, X_0 = 0.15	0.059 ± 0.009	11.8	1.94 ± 0.23	0.059 ± 0.004
10% ZM, X_0 = 0.4	0.081 ± 0.009	8.6	0.89 ± 0.04	0.040 ± 0.002
20% ZM, X_0 = 0.4	0.057 ± 0.021	12.2	1.27 ± 0.01	0.037 ± 0.001
Controlled conditions, open bioreactors				
10% ZM, X_0 = 0.15	0.102 ± 0.001	6.8	1.21 ± 0.07	0.047 ± 0.004
20% ZM, X_0 = 0.15	0.082 ± 0.003	8.5	1.55 ± 0.19	0.057 ± 0.003
10% ZM, X_0 = 0.4	0.053 ± 0.004	13.1	0.85 ± 0.01	0.039 ± 0.000
20% ZM, X_0 = 0.4	0.031 ± 0.002	22.4	1.07 ± 0.11	0.047 ± 0.001
Greenhouse conditions, closed bioreactors				
10% ZM, X_0 = 0.15	0.257 ± 0.001	2.7	0.91 ± 0.12	0.065 ± 0.001
20% ZM, X_0 = 0.15	0.322 ± 0.002	2.2	1.10 ± 0.08	0.094 ± 0.006
10% ZM, X_0 = 0.4	0.168 ± 0.037	4.1	1.00 ± 0.02	0.089 ± 0.006
20% ZM, X_0 = 0.4	0.215 ± 0.005	3.2	1.16 ± 0.04	0.112 ± 0.001
Greenhouse conditions, open bioreactors				
10% ZM, X_0 = 0.15	0.113 ± 0.017	6.1	0.83 ± 0.01	0.043 ± 0.008
20% ZM, X_0 = 0.15	0.160 ± 0.046	4.3	1.33 ± 0.06	0.054 ± 0.007
10% ZM, X_0 = 0.4[b]	0.153	4.5	1.32	0.077
20% ZM, X_0 = 0.4	0.146 ± 0.004	4.8	1.73 ± 0.04	0.094 ± 0.019

[a] Rounded to nearest day.
[b] One run only, no replicate.

creased as the quantity of inoculum increased. In fact, the lowest growth rate (μ_{max} = 0.031) of all the experiments occurred in open bioreactors under controlled conditions in 20% Zarrouk medium inoculated with 0.4 g L^{-1}, giving a biomass doubling time in excess of 22 d (Table I). Under greenhouse conditions the highest μ_{max} value was 0.322 d^{-1} in closed bioreactors containing water supplemented with 20% Zarrouk medium and inoculated with 0.15 g L^{-1} of *S. platensis* biomass, giving a biomass t_d value of 2.2 d, the most rapid growth rate of all the experiments (Table I). In the closed bioreactor greenhouse experiments μ_{max} decreased when X_0 was increased to 0.4 g L^{-1}, which might be caused by the high initial biomass concentration and could result in a more rapid depletion of nutrients. This hypothesis is reinforced by the fact that under greenhouse conditions μ_{max} in both types of reactors was highest for cultures supplemented with 20% Zarrouk medium.

When all other factors were constant, except Zarrouk medium content, cultures in water supplemented with 10% Zarrouk medium reached the death phase before those supplemented with 20% Zarrouk medium. In the greenhouse experiments involving closed bioreactors X_{max} was 1.16 g L^{-1} after about 17 d (Fig. 1), while in the open bioreactors under the same conditions X_{max} was 1.73 g L^{-1} after about 23 d (Fig. 1). In both cases the biomass concentration decreased rapidly after reaching the maximum values. Under greenhouse conditions, the X_{max} value for the closed bioreactors appeared not to be very influenced by the inoculum, while in the open bioreactors X_{max} was higher when the inoculum was 0.40 g L^{-1}. Independent of all other factors, the cultures supplemented with 10% Zarrouk medium presented lower biomass concentrations than those supplemented with 20% Zarrouk medium, which showed an average X_{max} value of 0.22 g L^{-1} for the closed bioreactors and 0.37 g L^{-1} for the open bioreactors (Figs. 1 and 2).

Regarding the maximum productivity (P_{max}), under controlled conditions in both the open and closed bioreactors this value was lower as the inoculum concentration increased (Table I). P_{max} in

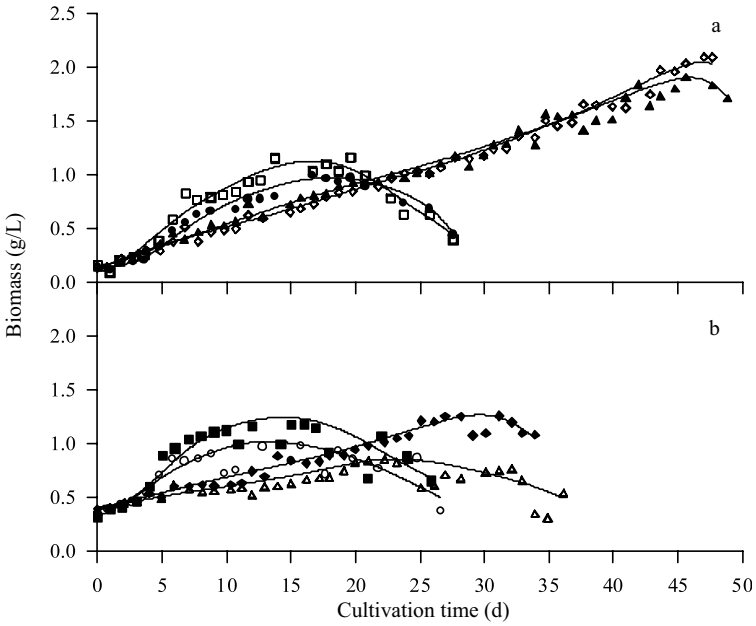

Fig. 1. Growth of *Spirulina platensis* strain LEB-52 under controlled conditions (CC) and greenhouse conditions (GC) in closed bioreactors containing water supplemented with different percentages of Zarrouk medium (ZM) and inoculated with different initial biomass concentrations (X_0, g L^{-1}) of *S. platensis*. (a) CC, 10% ZM, X_0 = 0.15 (▲); GC, 10% ZM, X_0 = 0.15 (●); CC, 20% ZM, X_0 = 0.15 (◇); GC, 20% ZM, X_0 = 0.15 (□). (b) CC, 10% ZM, X_0 = 0.40 (△); GC, 10% ZM, X_0 = 0.40 (○); CC, 20% ZM, X_0 = 0.40 (◆); GC, 20% ZM, X_0 = 0.40 (■).

cultures inoculated with 0.15 g L^{-1} (Table I) was reached after about 7 d and then stabilized at P = 0.04 g L^{-1} d^{-1}, while in cultures inoculated with 0.4 g L^{-1} P_{max} was reached after approx. 6 d and then declined to around 0.02 g L^{-1} d^{-1}. Vonshak *et al*. (1982) pointed out that it is economically important to maintain high productivities as the biomass concentration increases, which is not always the case during the growth of photosynthetic microorganism because the increased number of cells at high biomass concentrations not only reduces the osmotic pressure of the medium due to nutrient depletion but also results in a 'shading effect' which decreases the light available to individual cells. Contrastingly, in the greenhouse experiments P_{max} increased for both types of bioreactors as the inoculum concentration increased, with P_{max} occurring within 8 days and reaching an average of 0.112 g L^{-1} d^{-1} for the closed bioreactors and 0.094 g L^{-1} d^{-1} for the open bioreactors. When the content of Zarrouk medium was raised from 10% to 20% the increase in P_{max} was more accentuated in the greenhouse experiments than under controlled conditions, indicating that, when *S. platensis* is grown under greenhouse conditions, supplementation of water with only 10% of Zarrouk medium could be a limiting factor for productivity. In the first 4 days of cultivation there was a decrease in the productivities of all the greenhouse cultures (Figs. 1 and 2). This was possibly due to the fact that the temperature reached 46 °C in the greenhouse during this period, a value above the maximum reported value for the growth of *Spirulina* (Tomaselli *et al*., 1993).

Analysis of variance indicated that environmental conditions were the most important factor influencing the magnitude of the maximum growth parameters, the exception being the maximum biomass concentration (X_{max}) in the open bioreactors where the concentration of Zarrouk medium was the most important factor.

Solar illuminance reached 2800 μmol m^{-2} s^{-1} in the greenhouse experiments, a value much higher than the 32.5 μmol m^{-2} s^{-1} used in the controlled cultivations. The effects of the initial biomass concentration (X_0) on the growth of *S. platensis* suggest a relationship between X_0 and light penetration into the interior of the cultures. Increased biomass density can provoke shading in the interior of the medium (Vonshak *et al*., 1982), and in

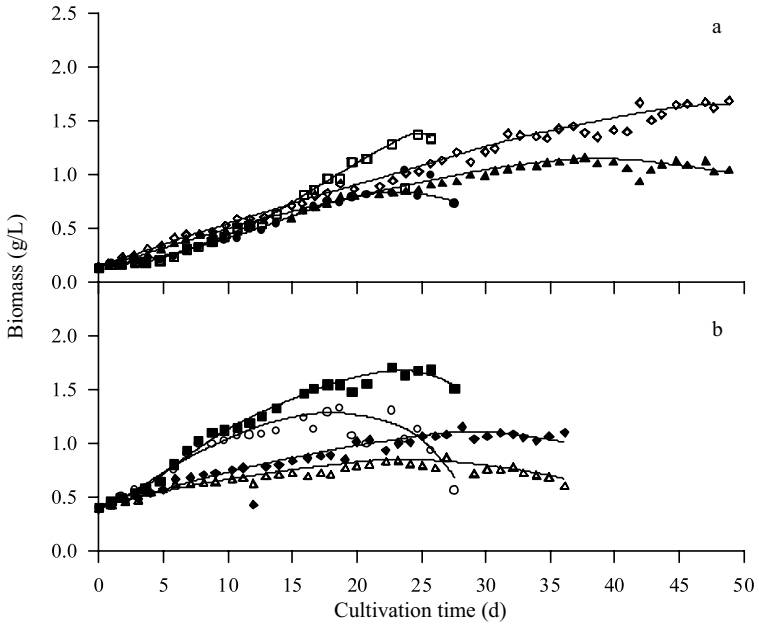

Fig. 2. Growth of *Spirulina platensis* strain LEB-52 under controlled conditions (CC) and greenhouse conditions (GC) in open bioreactors containing water supplemented with different percentages of Zarrouk medium (ZM) and inoculated with different initial biomass concentrations (X_0, g L^{-1}) of *S. platensis*. (a) CC, 10% ZM, X_0 = 0.15 (▲); GC, 10% ZM, X_0 = 0.15 (●); CC, 20% ZM, X_0 = 0.15 (◇); GC, 20% ZM, X_0 = 0.15 (□). (b) CC, 10% ZM, X_0 = 0.40 (△); GC, 10% ZM, X_0 = 0.40 (○); CC, 20% ZM, X_0 = 0.40 (◆); GC, 20% ZM, X_0 = 0.40 (■).

the high-luminosity greenhouse experiments shading could have reduced the penetration of light and decreased photoinhibition (Lu and Vonshak, 1999; Chanawongse *et al.*, 1994), while under the 32.5 μmol m^{-2} s^{-1} used under controlled conditions light could have been a limiting factor at high X_0 values due to photolimitation (Chojnacka and Noworyta, 2004).

In the greenhouse experiments temperatures varied from 9.4 to 46 °C, with the lowest temperatures generally occurring at night. Although the optimum growth temperature for *Spirulina* has been reported to be 30–38 °C (Belay, 1997), temperature variation during the light/dark cycle may result in higher productivities due to a drop in dark-phase respiration as a result of low temperature. Torzillo and Vonshak (1994) reported that the dark-phase respiratory activity of *Spirulina* increases with increasing temperature, reaching a maximum at 45 °C and consuming up to 35% of the biomass produced during the light-phase (Torzillo *et al.*, 1991). In the controlled cultures maintained constant at 30 °C dark-phase respiration would have been higher than in the greenhouse experiments, leading to higher respiration and hence lower net productivity.

The temperature and illuminance levels, and the variation in these factors, to which the greenhouse cultures were exposed, resulted in increased growth in both reactor types. However, this high growth rate appears to have stressed the cells and produced an earlier death phase (Figs. 1 and 2). This effect was more accentuated in the closed bioreactors where temperature change was the result of incident solar radiation and conduction through the glass walls of the bioreactors, resulting in high temperature in the liquid medium and thus in an unfavourable micro-environment for *S. platensis*. In the open bioreactors high temperatures were mitigated because heat flux between the culture medium and the atmosphere was facilitated by convection.

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